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INVERTEBRATE CONTROL AGENTS - EFFICACY TEST METHODS

VOLUME VII

HUMAN AND PET TREATMENTS

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16. Abstracts Testing procedures stated and referenced in this document are accepted methods for determining the effectiveness of pesticides against pests of humans and pets. The procedures mentioned are not intended to exclude other procedures. This document may serve as a guideline for the development of information relating to pesticide efficacy. Species named in this document are not necessarily the only ones which might be used. Other species may also be considered when of economic importance or when they provide practical data. When numbers of test animals are referred to in this document, the minimums are given. However, the researcher may want to increase numbers of test subjects, animals or other organisms when practical or economically feasible.

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biting flies
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ENVIRONMENTAL PROTECTION AGENCY

ANALYSIS OF SPECIALIZED PESTICIDE PROBLEMS
INVERTEBRATE CONTROL AGENTS - EFFICACY TEST METHODS
VOLUME VII
HUMAN AND PET TREATMENTS

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II

Report To The
Environmental Protection Agency

By The

American Institute of Biological Sciences
Arlington, Virginia 22209

EPA REVIEW NOTICE

This Report has been reviewed by the Office of Pesticide Programs, Criteria and Evaluation Division, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement of recommendation for use.

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HUMAN AND PET TREATMENTS

Table of Contents

		Page
1.	Human Treatments	2
1.1	Materials for the Control of Lice on Humans	2
1.11	The Body Louse	2
1.111	Infestation	2
1.112	Testing Procedures	2
1.112.1	Field Evaluation Of Insecticidal Powders	3
1.112.11	Data Reporting	3
1.112.12	Evaluation of Tests	3
1.112.2	Simulated Field Tests	4
1.112.3	Sleeve Tests with Pesticidal Powders	4
1.112.4	Tests with Impregnated Clothing	4
1.113	Insecticide Resistance in Lice	5
1.12	Head Lice and Crab Lice	6
1.2	Mites	7
1.3	Mosquitoes	8
1.31	Application to Skin	8
1.32	Application to Clothing	9
1.33	Application to Bed Nets, Head Nets, and Net Jackets	9
1.4	Biting Flies	12
1.5	Fleas	13
1.6	Ticks	15
2.	Pet Treatments	16
2.1	Fleas and Ticks	16
2.11	Dogs and Cats	16
2.111	Controlled Laboratory Testing	16
2.111.1	Number of Test Animals	16
2.111.2	Housing and Conditioning Animals	16
2.111.3	Preparation for Testing	16
2.111.4	Testing Considerations - Placebo	17
2.111.5	Testing Considerations - Standard	17
2.111.6	Testing Considerations - Water	17
2.112	Simulated Field Conditions	17
2.113	Consumer Field Testing	17
2.114	Test Arthropods	17
2.114.1	Ticks	18
2.114.2	Fleas	18
2.115	Infestation Methods	18
2.115.1	Ticks	18
2.115.2	Fleas	18

Table of Contents Continued

		Page
2.116	Observations	18
2.116.1	Qualifications	18
2.116.2	Pretreatment	19
2.116.3	Toxicity	19
2.117	Duration of Effectiveness	19
2.118	Types of Treatments and Testing Variations	19
2.118.1	Collars, Tags, Lockets	19
2.118.2	Types of Treatments	20
2.118.3	Systemics	20
2.12	Miscellaneous Other Animals	20
2.2	Lice	22
2.21	Dogs and Cats	22
2.22	Other Mammals	22
2.23	Birds	22
2.3	Mites	23
2.4	Biting Flies	24
2.5	Mosquitoes	24

INTRODUCTION

Testing procedures stated and referenced in this document are accepted methods for determining the effectiveness of pesticides against pests of humans and pets. The procedures mentioned are not intended to exclude other procedures. This document may serve as a guideline for the development of information relating to pesticide efficacy.

Species named in this document are not necessarily the only ones which might be used. Other species may also be considered when of economic importance or when they provide practical data. However, specie(s) used should be identified.

Because efficacy studies against a few human pests were discontinued after World War II and the Korean conflict, no recent studies are available. The war time data are still valid as the testing procedures have not changed. However, conducting efficacy studies against pests of humans has changed in that tighter restrictions have been placed on the use of human as test subjects.

When numbers of test animals are referred to in this document, the minimums are given. However, the researcher may want to increase numbers of test subjects, animals or other organisms when practical or economically feasible.

Generally control of a pest by a new product whould be as effective as that by an established commercial product, or the new product should have some other significant contribution such as safety or economy. There are many factors involved in actual use conditions that testing cannot always adequately evaluate. The habits of the host animals, the infestation pressures of the parasite, the exposure to rain, dew, sunlight or even the pH of water and other geographical considerations can result in a change in the degree or duration of efficacy for a particular product. Therefore, in order to make claims more meaningful, an accurate description of product performance under any of the varied conditions which may be encountered in the field should be recorded.

1. Human Treatments

1.1 Materials for the Control of Lice on Humans

Humans may be infested by three types of lice: the body louse, *Pediculus humanus humanus* L.; the head louse, *Pediculus humanus capitis* De Geer; and the crab louse, *Pthirus pubis* (L.). Pesticides that are effective against one species are usually effective against others, but this may not be true for new pesticides. The demonstration of effectiveness against any species or subspecies, if available, should therefore, be based on tests against lice of that particular type. Methods of application, and evaluation, differ according to the differences in the habits of these types of lice.

1.11 The Body Louse

1.111 Infestation

All stages of body lice infest the clothing, particularly the underwear, and heavy infestations may build up when the clothing is not laundered at frequent intervals. The eggs are attached to the cloth, not to the body hairs, and the nymphs and adult lice hide in seams and other protected parts of the clothing except when actually feeding through the skin.

1.112 Testing Procedures

Pesticide tests are most indicative of the practical effectiveness of control measures when they are conducted on groups of persons whose clothing, habits, and environments are typical of those of the populations in which the measures are intended for use. However, since body lice are vectors of typhus, relapsing fever, and other diseases (Pan American Health Organization 1973), experiments including controls and pesticides of unproven effectiveness should be made only with infestations of lice that are known to be free of such diseases. Although toxicological clearance of new pesticides must precede field experimentation, such experiments should also be utilized for surveillance for potential physiological effects (e.g. cholinesterase inhibition) under conditions approximating the anticipated actual use.

In field experiments with naturally infested populations it is rarely possible to obtain all the conditions or observations that would be desired in an ideal evaluation of a pesticide treatment. Investigators should be alert to certain problems that are likely to be encountered in experiments with naturally infested populations. Even persons who ignore body lice under normal conditions may become sensitive to their presence under experimentation, and pick the lice from their clothing between observations. Some persons who give every assurance of full cooperation may

secretly attempt to remove the treatment from their clothing, and others may add such additional treatments as may be available to them.

Pesticides for the control of body lice have usually been incorporated in dusting powders, but the impregnation of clothing has also been used (Pan American Health Organization 1973).

1.112.1 Field Evaluation of Insecticidal Powders

Some methods used in field evaluations of insecticidal powders were given by Eddy (1952) Barnes et al. (1962), and Steinberg et al. (1971). Although the procedures may differ, they include essential features required to produce acceptable data.

1.112.11 Data Reporting

An adequate description of the technique includes pesticides used, powder carrier, pesticide concentration, dosage applied per person or per garment, application method (such as application to removed clothing or to clothing being worn by the test subject), sites of application (neck, sleeves, etc), the habit of the subjects, records of any medical treatments received by the subjects while under test, and history of known allergic reactions of the subjects.

Groups of subjects treated with different materials should be as uniform as possible in all respects. Groups including adult males only are satisfactory. At least one group should be treated with each experimental pesticide, one group with a standard pesticide of known effectiveness to demonstrate the suitability of the testing conditions, and one group (the control) with a placebo consisting of the carrier powder without a pesticide.

1.112.12 Evaluation of Tests

Efficacy of the treatment is based on the numbers of lice per person before treatment and at intervals after treatment. If the original infestation is small, counts may be based on all the lice found in the clothing, or on the number which can be found in a unit period of time if the infestations are heavier. Other methods of assessment might also be devised. The same methods should be used for counting lice at intervals after treatment (3, 7, 14, 21 days, etc). The degree of control obtained may be based entirely on the reduction of lice on the treated subjects if infestations in the control group remain uniform, or adjusted to compensate for changes in the control group (Abbott 1952). It is desirable that the treatment kill lice as quickly as possible, preferably within 24 hours. However, it is often impractical to make field observations of large groups of people within 24 hours after treatment, and the speed of action of the insecticides can be demonstrated in laboratory tests. The length of time

the powders remain effective is important in protecting against reinfestation, hence the observations should be continued over several weeks to determine duration of effectiveness. Unless the pesticides are ovicidal, extremely young nymphs may appear briefly even on effective treatments.

1.112.2 Simulated Field Tests

Simulated field test methods in which subjects wearing treated clothing are artificially infested with lice after various periods of wear have been conducted (Bushland et al. 1944). Experiments of this type are designed to approximate the expected conditions of actual use as closely as possible, except that the reinfestation pressure is precisely controlled. The reporting requirements are the same as those in section 1.112.11.

1.112.3 Sleeve Tests with Pesticidal Powders

Tests with treated sleeves worn by human subjects provide information approaching that obtainable in field tests as to the duration of effectiveness. They actually provide better information than that obtainable in field tests on quickness of kill, and are helpful in the clearance of compounds for field trials. They have the advantage of permitting a balanced incomplete block test design, since experimental compounds can be compared with standards on opposite arms and legs of the same subject under identical conditions, and reinfestation pressures are precisely though artifically simulated. This is the ideal stage for establishing the minimum effective concentration.

In the sleeve technique the powders are applied to a 1-sq. ft. inside area of cloth sleeves. The sleeves are fastened to the arms and legs of research subjects, worn for various periods of time, and infested with known numbers of lice after fixed periods of wear. The mortality of the lice is usually determined after 24 hours, but can be determined after shorter periods. In some tests with this method, the sleeves have been worn continuously for periods ranging up to 28 days (Bushland et al. 1944 , Cole and Clark 1962), and the lice were introduced into the sleeves while they were on the human subject. Comparable results in terms of duration of effectiveness were obtained when the sleeves were worn 12 hours per day for 4 days each week and tested off the host at weekly intervals (Cole et al. 1967, Hirst et al. 1970).

Reports of tests by this method should specify the pesticides, concentration, amount of powder per unit area, method of application, periods of wear between exposures of lice, number of lice of each stage or sex used, and the percent mortality.

1.112.4 Tests with Impregnated Clothing

Clothing can be impregnated with pesticides for protection from body lice (Bushland et al. 1944, Pan American Health Organization 1973). Reports

of test techniques and results with this method of application are similar to those for tests with powder treatments. The information should include specifications regarding the formulation used, the method of impregnation, and the amount of actual pesticide picked up by the treated garments.

1.113 Insecticide Resistance in Lice

In certain areas body lice have developed resistance to DDT, lindane, and other pesticides. Consequently, it is necessary to determine whether the strains of lice being used are resistant to the standard pesticides, and whether this has induced cross resistance to the experimental pesticides. Such determinations should be made in controlled laboratory tests by means of established resistance-test procedures, (Cole et al. 1957, 1973; Steinberg et al. 1971; Pan American Health Organization 1973).

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1.12 Head Lice and Crab Lice

Materials that will control body lice often are effective against head lice and crab lice, although the methods of application may differ. Since head lice and crab lice remain on the head or body hairs, rather than on the clothing, and attach their eggs to the hairs, liquid preparations are frequently preferred over powders. Evaluation of pesticides for the control of these species requires the same items of information as those mentioned for body lice (Section 1.11) and as given by Cowan et al. (1947) and Eddy (1948). Attention should be given to the age and sex of the subjects, previous pesticide treatments, concurrent drug treatment, and environmental conditions. The degree of infestation before or after treatment is based on the numbers of nymphal and adult lice, not on the presence of eggs, since even dead or hatched eggs remain on the hair for several days. The residual effectiveness of the treatment and the necessary intervals between retreatments should be determined.

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1.2 Mites

Several species of mites attack humans, but the only groups for which pesticides or repellents are regularly applied to the skin or clothing are the itch mite, *Sarcoptes scabiei* (De Geer), and the larvae of trombiculid mites known as chiggers or redbugs. Mites that are parasites of rodents or birds may infest houses and become human pests. These include the tropical rat mite, *Ornithonyssus bacoti* (Hirst) and the house mouse mite, *Allodermanyssus sanguineus* (Hirst), which are controlled by premise treatments.

Materials for protection from chiggers are most widely used by application to the clothing. These materials may sometimes be referred to as repellents because of their chemical identity with mosquito repellents. However, usually they function against mites as knockdown agents or toxicants. They may provide protection against other mites as well as chiggers. Many materials applied to the clothing will withstand some washing and still remain effective (USDA 1967). Evaluations of new materials should include a determination of the amount of washing the treatments will withstand. However, even materials which do not withstand washing may be highly useful for protection from chiggers if they are effective when freshly applied.

Field tests under practical or simulated practical conditions provide the best evaluation of chigger toxicants. These materials may provide adequate protection when applied to limited parts of the clothing (socks, trouser cuffs, waist band, and other openings) but final evaluations are usually based on the impregnation of all outer clothing.

Methods for the field evaluation of outer clothing (Snyder and Morton 1946, Cross and Snyder 1948) should specify: the compounds used, garments treated, method of impregnation, impregnating formulation, amount of toxicant deposited per garment or per unit area of cloth, aging period or the amount of washing or wear, method and period of exposure to chiggers, and the interval between the exposure and the counting of attached chiggers. The most conclusive indications of effectiveness are obtained when the treatment is compared to a standard treatment and untreated clothing.

Treated sleeves may be used to obtain preliminary or supplemental information (Cross and Fye 1949). This method has the advantage of providing direct comparisons between materials on opposite limbs of the same subject exposed at the same time.

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1.3 Mosquitoes

Materials applied to humans for protection from mosquitoes are usually repellents rather than insecticides.

Evaluation of mosquito repellents for practical use is based on the length of time they remain effective (USDA 1967). Mosquito repellents are applied to the skin, to clothing, or to wide-meshed netting for use in bed nets, head nets, or jackets.

1.31 Application to the Skin

The principal method used in the evaluation of mosquito repellents is the application of the material to the skin of the forearm or sometimes the lower leg. These areas are then protected from rubbing and exposed to mosquitoes in the laboratory or the field, either continuously or at specified periods, to determine the length of time the treatment provides complete protection or a high level of protection (Bacot and Talbot 1919, Granett 1940, Granett and Haynes 1945, Christophers 1947, McCulloch and Waterhouse 1947, Travis et al. 1949, Gilbert et al. 1957, Gerberg 1966, USDA 1967, Weaving and Sylvester 1967, and Altman 1969). Liquid repellents are frequently tested at full strength. The dosage most commonly used is 1 g applied evenly to the forearm or 1.5 g applied to the leg between the ankle and the knee. In laboratory tests the treated limbs are exposed to caged mosquitoes for 2 to 5 minutes at intervals of 30 to 60 minutes, whereas field tests require continuous exposure. The protection time is considered to be the time interval between treatment and the time required to receive the first confirmed bite, or the time to receive 2, 5 or more bites. However, actual protection times vary widely with differences in the hosts, the avidity of the mosquitoes, and the environmental conditions; therefore the most reliable criterion of effectiveness is the ratio of the protection time of the experimental repellent to that of a standard repellent tested at the same time on the opposite limb of the same subject. If several experimental repellents are to be tested at the same time, the number of replications of the standard can be reduced by the use of an incomplete block design (Gilbert et al. 1955, Gilbert 1957, Smith 1958).

If protection times are impractically long it may be necessary to use dilutions of the repellents in alcohol or other solvents (Gilbert et al. 1955, 1957, Altman and Smith 1955, Altman 1969).

Repellents vary in the amount per unit area of skin required to provide protection under any given set of conditions (minimum effective dose). The protection time represents the time required for the repellent to reach a minimum effective dose. When skin is protected from rubbing, the modes of loss are evaporation and absorption (Smith et al. 1963). When repellents are used under conditions that induce sweating the rate of loss is greatly increased, and complete evaluation of new repellents should include comparisons with a standard under sweating conditions in laboratory or field tests (Gilbert et al. 1957).

In actual use, much of the repellent is lost from the skin by rubbing against the clothing or other objects, and the protection time is greatly reduced. Determining the resistance of a repellent to removal by rubbing or wiping is, therefore, an important part of its evaluation (Christophers 1947, Smith 1958).

Repellents vary in effectiveness against different species of mosquitoes, and a complete evaluation should, therefore, include tests against a variety of species. *Aedes aegypti* (Linnaeus) is the species most commonly used in the laboratory, although several species of *Anopheles* are frequently used. Field tests should range over various species of *Aedes, Anopheles, Psorophora, Mansonia,* and *Culex.*

1.32 Application to the Clothing

Evaluations of materials as clothing impregants should specify the following: repellent used, impregnating formulation, method of impregnation, garments treated, amount of repellent absorbed per unit area of garment, interval between treatment and each exposure to the mosquitoes, method an conditions of exposure, and the number of bites received per unit of time (e.g., Smith and Cole 1951, Smith and Gilbert 1953, Gilbert et al. 1957, Traub and Elisberg 1962). The efficacy of test repellents is most indicative when compared to that of standard repellents.

1.33 Application to Bed Nets, Head Nets, and Net Jackets

Repellents may be used to treat wide-mesh netting used for bed nets, head nets, or loose jackets. Although laboratory studies are useful in the selection of compounds for full evaluation, definitive demonstrations of the practicality of the treatments depend on evaluations of their effectiveness under field conditions. Methods used in the field evaluation of repellent-treated netting under practical conditions or simulated practical conditions have been described by Gouck et al. (1967, 1971), Hirst et al. (1968), Gouck and Moussa (1969), Grothaus et al. (1972, 1974), and McDonald and Grothaus (1973).

Reports of field tests with treated netting should include the: type of netting (the type of fiber is important because cotton absorbs repellents to a much greater extent than synthetic materials), mesh size and weight per unit area of netting, impregnating formulation, method of impregnation, amount of repellent absorbed per unit area or weight of netting, construction of the experimental item (e.g., bed net), and method of exposure. In general, the subjects protected by treated netting should be compared to subjects protected by untreated netting as well as to subjects not protected by netting. Efficacy is determined by the numbers of mosquitoes penetrating the nets, biting the protected subjects, and biting the unprotected subjects in a specified exposure period. Mosquitoes should be identified to permit evaluation of the effectiveness against different species. Record the time between treatment and subsequent exposures, method of storage between exposures, and any adverse conditions between exposures (e.g., the amount of rainfall) to which the treated nets are exposed. Comparisons of experimental repellents with a standard repellent are advisable.

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1.4 Biting Flies

Methods of evaluating repellents applied to humans for protection from biting flies are similar to those described for the evaluation of repellents against mosquitoes (Applewhite and Cross 1951, Gilbert et al. 1957, Gerberg 1966). Behavioral variations between species may necessitate minor adjustments in techniques; for example, deer flies *(Chrysops* spp.*)* are attracted to moving objects and test subjects usually remain in motion with upraised arms during exposures to these species (Gilbert et al. 1957, Schreck et al. 1976). The intensity of sunlight is also a factor.

Although black flies (Simuliidae) may bite viciously in some localities, evaluations in other localities have been based on a long period of protection without bites rather than protection time to the first bite (Travis et al. 1951). The tendency of black flies and snipe flies, Rhagionidae, to alight on the shirt and then crawl over the edges of the sleeves onto skin untreated by repellent should also be taken into account in evaluations against these pests.

Biting midges *(Culicoides* spp.*)* present problems in evaluation as they may be extremely active for a few hours and then cease activity completely. Under these conditions a determination of relative protection periods requires treatment several hours in advance of the anticipated activity of the midges. These midges also have a tendency to land on treated skin even when the repellent is adequate to prevent actual biting (Applewhite and Cross 1951).

The methods used in the evaluation of repellent-treated bed nets and net jackets against various types of biting flies are also similar to those used in evaluations against mosquitoes (Frommer et al. 1975, Mulrennan et al. 1975, Sholdt et al. 1975).

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1.5 Fleas

Humans may become infested with many species of fleas (Hubbard 1947, Holland 1949) but are more commonly infested with fleas from hosts with which they are closely associated. These fleas are the cat flea, *Ctenocephalides felis* Bouche), and the dog flea, *C. canis* (Curtis), but may be the oriental rat flea, *Xenopsylla cheopis* (Rothschild), or the human fleas, *Pulex irritans* and *P. simulans* (Linnaeus), which are very important in cities and towns of the Southwest and occasionally become abundant on farms in the Midwest and Southern U.S. Many people are bitten by small insects popularly known as "sand fleas". In the North, these are usually the cat or dog fleas. In the West, they may be the human flea, sometimes associated with squirrels or prairie dogs whereas in the South, they may be the sticktight flea *Echidnophaga gallinacea* (Westwood), but are usually the dog and cat fleas. Flea control is usually divided into two categories: control on pets or premises treatments (see analysis of Specialized Pesticide Problems: Invertebrate control agents, Vol. V) and control on rodents for disease prevention.

Materials for human protection may be applied to the skin as repellents (Lindquist et al. 1944, Travis et al. 1946, Bar-Zeev and Gothilf 1972), but in areas of heavy flea infestation, clothing treated with repellents is preferable and affords better protection (Linduska et al. 1946, Smith and Burnett 1948, Gilbert et al. 1957). Initial testing should be conducted on humans as described in the literature cited above. In general, a repellent is effective against more than one pest of humans and therefore the screening and testing methods will be similar to that described in sections 1.111 - 1.114, 1.2 and 1.3.

Unfed fleas at least 12 hours old should be used in testing programs. Dog fleas, cat fleas, or Oriental rat fleas (*Xenopsylla cheopis* Rothschild) are useful laboratory test organisms; however, field testing should be conducted against fleas endemic to the area.

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1.6 Ticks

Several species of ticks occur on humans. These represent soft ticks (Argasidae) which in the nymphal and adult stages feed for brief periods only and the hard ticks (Ixodidae) which feed for several days and may react differently to pesticides. Two species that personal insect repellents are effective against are the lone star tick, *Amblyomma americanum* (L.) and the American dog tick, *Dermacentor variabilis* (Say).

Tick repellents for use on skin and clothing can be tested by procedures similar to those described for mosquitoes (Sections 1.3, 1.31, 1.32 and 1.33) and chiggers (Sections 1.2 and 1.21).

Selected references for testing tick repellents are provided.

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2. Application to pets

2.1 Fleas and ticks

2.11 Dogs and cats

Dogs and cats are usually infested with fleas and ticks although ticks occur more commonly on dogs.

Methods of testing the efficacy of pesticides against these pests are similar for both the dog and the cat, therefore, the method described below can be interchangeable for either animal.

2.111 Controlled Laboratory testing

Animals should be selected for testing based upon the following.

2.111.1 Number of test animals

At least six (6) animals per treatment (control animals are considered a treatment) with equal numbers of each sex and divided as equally as possible between the various factors as to breed, approximate age, weight, and hair coat, i.e., length and texture. These variables must be recorded.

2.111.2 Housing and conditioning animals

Housing conditions for animals vary (Section 2.118). However, animals should be housed in individual pens if at all possible. For dogs the holding environment should provide outdoor and indoor exposure.

Untreated animals should be housed in the same general area but away from the treated animals by at least 8 to 10 feet.

All animals should be properly conditioned for at least 7 days prior to testing. To assure survival during the test period all animals should be immunized during the conditioning period. All records of any medication administered to the animals during the course of the study should be recorded. Avoid using disinfectants for cleaning during the testing period. Other pesticides should not be used in or around the test area during the study.

2.111.3 Preparation for testing

All animals should be checked for natural infestation of the arthropod to be tested. These parasites should be counted (see Section 2.115) and some removed for proper identification. Animals not having a satisfactory infestation should be infested 2 days prior to treatment to allow proper

adjustments to the host. Not all individual animals are susceptible to arthropods. Those that will not take an infestation should be replaced with susceptible animals.

2.111.4 Testing Considerations - Placebo

A placebo treatment containing all ingredients except the pesticide should be administered to the untreated (control) animals.

2.111.5 Testing Considerations - Standard

It is advisable to run a registered treatment as a standard as an aid in evaluating the efficacy of the test product.

2.111.6 Testing Considerations - Water

Depending upon label claims, the effect of water wetting the animals versus the treatment should be established and recorded.

2.112 Simulated field conditions

The conditions which would be most likely to result in pest infestation in the field can be duplicated under a controlled research project (Knapp 1976).

2.113 Consumer field testing

When adequate data are obtained from laboratory and/or simulated field testing, additional regional field testing by consumers or by veterinarians is desirable. All of these tests should be supervised by a professional entomologist experienced in the specific testing area.

In these types of tests, it is important that an equal number of consumers receive an unknown placebo treatment.

Data to be recorded should be the geographical region; treatment date; environmental conditions; the hosts' age, breed, sex, hair (length and texture); health conditions (medication and other pest treatment received prior to or during the course of the test); habits (for example, free to roam, yard pet, house pet, sleeping area, etc.); and the pest organism being controlled and information as to the reinfestation pressures. At least 20 acceptable hosts should be utilized in each field or consumer geographical testing region. The comment of the owner is often useful information in judging the effectiveness of a product.

2.114 Test arthropods

2.114.1 Ticks

Unfed adult ticks are recommended for testing. When ticks are in great numbers in the area they can be trapped using CO_2 traps (Semtner and Hair 1975) or using a flannel cloth drag. Another method of collecting is to remove the unengorged adult ticks from stray dogs and use these to infest the treated dogs. When natural tick populations are not available, they can be laboratory reared (Gregson 1966 and Srivastava and Varna 1964).

2.114.2 Fleas

The dog flea and cat flea interchange host readily, and are equally susceptible to most pesticides. Therefore, data derived on one species will support the claim for the other species. However, the species used in the study should be reported.

2.115 Infestation methods

2.115.1 Ticks

All tested dogs, including those untreated, should be infested with a minimum of 25 ticks each. This should be accomplished by placing unfed, 10 to 14 day old adult ticks in the housing or sleeping area of the dog. This allows the tick to become attached in a natural way. Ticks can also be deposited directly onto the dog's back as the hair is ruffled with the hand by a forward brushing motion. Some ticks will attach immediately but most will crawl over the body to seek a site for attachment. Reinfestations should be done 2-3 days prior to each post treatment observations.

2.115.2 Fleas

Cat or dog fleas can be easily reared in the laboratory (Smith and Eddy 1954, Hudson 1958). Infestations of the cat or dog can be accomplished by depositing 50-100 unfed adult fleas either directly onto the animal or in the bedding or by placing flea pupae in a holding container in the housing area and allowing the adults to seek the host upon emergence. If the pupal exposure method is used, care should be taken to assure emergence of the fleas 2 days prior to the post treatment counts. Reinfestations should be made 2 days prior to each post treatment count.

2.116 Observations

2.116.1 Qualifications

Satisfactory observations require qualified personnel supervised by a professional entomologist.

2.116.2 Pretreatment

A pretreatment count of test arthropods should be made immediately prior to treatment and then periodic counts are made throughout the study, depending upon the degree of time the treatment is expected to last. (See Section 2.117).

These inspections should indicate the number of test organisms and sex found and in case of ticks whether attached or crawling; the viability (living or dead) and area of the body in which found. These data should be recorded as days after treatment started and compared to counts found on the untreated and the standard treatment if one is used.

All ticks found should be counted. It may not be possible to count all fleas and perhaps a rating system such as light (1-25), med. (26-50) and heavy (50 or more) can be used for the flea counts.

2.116.3 Toxicity

All toxic symptoms of the host animal during the course of the study should be noted and recorded. If death occurs a post mortem report is useful in clarifying cause of death.

2.117 Duration of Effectiveness

The greatest importance is the degree of control afforded by the treatment. Such claims as "controls" must be supported by data which indicate that the treatment will effectively reduce substantial populations of pest arthropods to a level equal to or greater than comparable commercially used products. For all practical purposes, this would be control to the extent that the user would not usually be required to rely on other pesticides or methods of control during the time period specified on the labeling.

2.118 Type of treatments and testing variations

2.118.1 Collars or medallions (tags, lockets, etc.)

There are many commercially produced impregnated materials for the control of ticks and/or fleas on pets. The general testing procedures to obtain the data acceptable for label claims have been presented in sections 2.111 - 2.117. There are many variations to these procedures. For instance some flea researchers confine cats to closed individual housing and vary in their infestation rate and methods (Fox et al. 1969 and 1969a, Knapp 1976). Others use dogs under natural conditions (Knapp 1976, Quick 1971, Kalkofen and Greenberg 1974, 1974a) and controlled conditions (Knapp 1976 and Kibble 1968). All of the above methods cited have produced reliable data.

Information on reinfestation rates with impregnated collars or medallions is not usually needed during the early weeks of the test unless the control claim is less than 1 month. Post treatment infestations for long-lasting collars can be done at 1, 2, and 3 months and at weekly intervals thereafter to establish the duration of effectiveness. Usually when control drops below 80%, the test is terminated. All test animals should be wetted at least monthly. Records should be kept of any abnormalities to the host due to the treatment such as neck rashes.

2.118.2 Shampoos, dips, sonnges, aerosols, sprays, dusts, spot-treatments.

Various commercial products containing pesticides are available for tick and flea control. Test procedures for these products are essentially the same as described in sections 2.111 - 2.117 with the exception that flea and tick counts are made more frequently due to the short residual effectiveness of the treatment. (Lindquist et al. 1944, Sweetman 1946, Hansen 1956, Hopwood and Migden 1965, Stampa 1970, Butt 1971, and Knapp 1976).

2.118.3 Systemics

Some systemic insecticides are available to veterinarians for administering to pets for external parasite control. These are considered drugs but the initial test procedures are essentially the same as those of Hill et al. (1963) and Clark et al. (1971).

2.12 Miscellaneous other animals

Fleas and ticks attack other animals such as commercial fur-bearing animals and zoo animals. A few exotic pet animals are also subject to becoming infested with fleas and ticks.

There is no testing protocol established on these animals but if one wishes to establish a label claim for one species or a general claim for several animals then the guide lines established for dogs and cats may be followed probably with only few modifications. The guide lines established for testing pesticides against livestock pests may be helpful, especially for the larger, closely related, zoo animals.

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2.2 Lice

2.21 Dogs and Cats

In the United States dogs are infested by two lice, *Trichodectes canis* (De Geer) and *Heterodoxus spiniger* (Enderlein) whereas cats are parasitized by three, *Felicola subrostratus* (Burneister), *Tricholipeurus liperuroides* (Megnin) and *T. parallelus* (Osborn). However, dogs and cats infested by these lice in any great quantities are rare. Usually lice on dogs or cats are controlled by the same treatments approved for fleas or ticks (Sweetmen 1946).

Tests for conducting efficacy studies of pesticides against lice on dogs and cats can be similar to those for livestock (Babcock 1944) or similar to the methods for fleas and ticks (Section 2.1). However, tests should be conducted in areas endemic to the infestation of the dog and cat lice.

2.22 Other mammals

Guinea pigs and other small mammals as well as coyotes, wolves, larger zoo animals and exotic pets (Emerson 1962a) may become infested with lice. Efficacy studies similar to those discussed in Sections 2.1 and 2.31 can be used to evaluate pesticides for their control.

2.23 Birds

Usually only chewing lice (Mallophaga) infest birds (Emerson 1962). They are found in all areas of the body but certain species may be more prevalent in specific areas than other species. In general, lice on domestic pet birds are not a serious problem. However, the pigeon has two lice, *Companulates bidentatus* (Scopoli) and *Columbicola columbae* (Linnaeus) which live among the feathers of both old and young birds and interfere with profitable raising of these fowls. Usually the control methods used for domestic fowls have been adopted for use on other birds. Tests should be conducted in areas endemic to the infestations.

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2.3 Mites

Mites cause injury to small animals, especially dogs and cats. Probably most important is sarcoptic acariasis or mange. The common mange of dogs is caused by *Sarcoptes scabiei* var. *canis* (Gerloch). Mange of cats is caused by *Notoedres cati* (Hering). Auricular mites cause otoacariasis of cats and dogs. These mites literally swarm in the ears of their host. The rabbit fur mite, *Cheyletiella parasitivorix* (Megnin), is occasionally found on cats and dogs. Symptoms include the loss of hair, cracked skin, loss of appetite and extreme discomfort.

Testing procedure can generally follow that outlined under 2.1 to 2.114 in this section.

Ear mites require more specialized treatment. Controlled evaluation provides the most useful data (see Tonn 1959); but field testing is also necessary. Infestations vary greatly from animal to animal (approximately 20) as possible to insure that uniform numbers of mites are used in the tests. Frequently less than 50% of dogs will have adequate numbers of mites for use in evaluation. Working dogs which have contact with other animals and are exposed to variations in the environment provide better data than controlled dogs. The same situation is true for cats.

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2.4 Biting Flies

Small animals, especially dogs and cats, are bothered by biting flies of the same species that attack humans and livestock. Species that attack dogs include stable flies *(Stomoxys calcitrans)*, horn flies *(Haematobia irritans)*, biting midges *(Culicoides* spp.) and black flies *(Simuliidae)*. Shortness of hair increases annoyance to these animals. The face and ears of dogs are especially attractive to biting flies. These areas require special attention and they are difficult to treat because repellents do irritate mucus membranes.

Repellents normally acceptable for use on humans and horses may be used on dogs. There is no testing protocol established for these animals.

2.5 Mosquitoes

Repellents are applied to dogs and to a lesser extent to cats for protection from mosquitoes. Mosquitoes that attack humans also feed on dogs and cats and other small animals. The same guidelines noted for biting flies (2.4) can be applied for testing against mosquitoes.